

Claims

1. A method for identifying and/or isolating mycolic acid bacterial DNA encoding an inducible promoter which is induced in response to a specific analyte and/or associated operon proteins, the method comprising the steps of:

- (a) culturing a source of mycolic acid bacteria in a selective medium containing said specific analyte and being selective for oligotrophic bacteria,
- (b) identifying mycolic acid bacteria capable of subsisting on said medium,
- (c) extracting DNA from said mycolic acid bacteria,
- (d) incorporating said DNA into a vector,
- (e) cloning said vector into a suitable host cell, and
- (f) screening the host cell for said inducible promoter and/or proteins in order to identify vectors encoding it.

2. A method as claimed in claim 1 wherein the analyte is an environmental pollutant.

3. A method as claimed in claim 2 wherein the environmental pollutant is a hydrophic organic compound.

4. A method as claimed in <sup>claim 1</sup> ~~any one of the preceding claims~~ wherein the mycolic acid bacterium is a member of the Rhodococcus or Nocardia complex.

5. A method as claimed in <sup>claim 1</sup> ~~any one of the preceding claims~~ wherein the medium used in step (a) comprises less than <500 µM carbon supplement.

6. A method as claimed in <sup>claim 1</sup> ~~any one of the preceding claims~~ wherein the mycolic acid bacteria isolates are screened after or during step (b) to ensure an absence of catabolic repression.

7. A method as claimed in claim 6 wherein the catabolic repression screen is performed by assessing the concentration of an enzyme associated with the specific analyte of interest in (i) medium supplemented with the specific analyte, and (ii) medium supplemented with the specific analyte plus a high efficiency carbon source, and (iii) medium not containing the specific analyte but containing a high efficiency carbon source.

8. A method as claimed in any one of the preceding claims wherein the mycolic acid bacteria are grown on a medium comprising L-glycine prior to the DNA extraction at step (c).

9. A method as claimed in claim 8 wherein the mycolic acid bacteria are washed using 0.05 - 0.5 % (v/v) non-ionic detergent prior to the DNA extraction at step (c).

*a* 10. A method as claimed in <sup>Claim 1</sup> ~~any one of the preceding~~ claims wherein the host cell of step (e) is an E coli strain carrying one or more of the mcrABC, mrr, hsdSRM, recA or recO mutations. *a* *C*

11. A method as claimed in any one of the preceding claims wherein the host cell is screened for a sequence comprising an inducible promoter and/or operon proteins by using one or more oligonucleotide probes or primers corresponding to, or complementary to, a promoter and/or operon protein derived from a mycolic acid bacterium and selecting vectors which are complementary to, or specifically hybridisable with, said probe or primer.

12. A method as claimed in claim 11 wherein the oligonucleotide probe or primer comprises a sequence of at least 20, 30, 40, 50, or 100 nucleotides, said sequence corresponding to, or being complementary to, all or part of a contiguous sequence of the R. corallina ohp

operon.

13. A method as claimed in ~~any one of claims 1 to 10~~ wherein the host cell is screened by:

(i) incorporating a sequence believed to comprise an inducible promoter plus optionally further operon proteins in a vector at a position in which it is operatively linked to a coding sequence,  
(ii) transforming a host cell with said vector, and  
(iii) determining the presence or absence of the coding sequence expression product in the presence of the analyte.

14. As method as claimed in any one of claims 1 to 10 wherein the host cell is screened for the inducible promoter and/or operon proteins by screening for an activity associated with the inducible promoter and/or operon proteins.

15. A method as claimed in claim 14 wherein the activity is an enzyme activity for which the analyte is a substrate.

16. A method as claimed in claim 15 wherein the enzyme activity is screened for by contacting the host cell or an extract thereof with a substrate for the enzyme and observing the cell or extract for enzymatically generated products of the substrate.

17. A method as claimed in any one of claims 14 to 16 wherein the vector is transferred from a first host cell of step (e) to a second host cell wherein the activity is screened.

18. A method as claimed in claim 17 wherein the second host is a mycolic acid bacterium.

19. A method as claimed in claim 18 wherein the second host is a Corynebacterium.

20. A method as claimed in <sup>claim 16</sup> ~~any one of claims 17 to 19~~ wherein the vector is transferred from the first to the second host by bacterial conjugation.

21. A method as claimed in any one of claims 17 to 20 wherein the vector is shuttle vector capable of replication in the first and second hosts.

22. A method as claimed in claim 21 wherein the vector comprises two, three, four or five of the following elements: (i) a replicon for mycolic acid bacteria; (ii) a replicon for E. coli; (iii) a conjugative origin of transfer; (iv) a lambda cos site; (v) a sequence encoding an antibiotic marker gene.

23. A method as claimed in claim 22 wherein the elements are selected from a group comprising: pCY104oriV; pBR322 oriV; RP4 oriT; pSR1.

24. A method as claimed in claim 23 wherein the plasmid is selected from: pJ8; pRV1; pJH6 as described herein.

25. A method of producing a modified inducible promoter and/or operon, the method comprising the step of modifying a nucleotide sequence encoding the inducible promoter and/or operon identified in accordance with the method of <sup>claim 1</sup> ~~any of the preceding claims~~.

26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an inducible promoter and/or operon protein identified in accordance with the method of <sup>claim 1</sup> ~~any one of claims 1 to 24~~ or produced by the method of claim 25.

27. A nucleic acid as claimed in claim 26 comprising a promoter region of the nucleotide sequence encoding the R. corallina ohp operon described in Figure 3.

28. A nucleic acid as claimed in claim 26 encoding one or more of the following proteins of the R. corallina ohp operon: Regulator REG; Transport TRANS; Monooxygenase MONO; Hydroxymuconic semialdehyde hydrolase HMSH; Alcohol dehydrogenase ADH; and Catechol 2, 3-dioxygenase CDO.

29. A nucleic acid molecule comprising a sequence encoding a modified inducible promoter obtainable by the method claim 25 which is at least 70%; 80%; 90%; 95% or 98% identical to the sequence of the inducible promoter of claim 26 ~~or claim 27~~.

30. A nucleic acid as claimed in <sup>Claim 26</sup> ~~any one of claims 26 to 29~~ further comprising a heterologous signal gene.

31. A nucleic acid comprising (a) a sequence capable of effecting site specific integration of a heterologous signal gene into the genome of host cell such that it is operably linked to an inducible promoter identified in accordance with the method of <sup>Claim 1</sup> ~~any one of claims 1 to 24~~; (b) a heterologous signal gene.

32. A vector comprising the nucleic acid of claim 30 ~~or claim 31~~.

33. A vector as claimed in claim 32 comprising one or more of the following: luxAB signal genes; sacB gene; antibiotic resistance; RP4/RK2 mobilizing elements.

34. A vector as claimed in claim 33 which is pJP7 as described herein.

35. A method of transforming a host cell comprising use

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of a vector as claimed in <sup>claim 32</sup> ~~any one of claims 32 to 34~~.

36. A method as claimed in claim 35 wherein the host cell is transformed by site specific integration such that the signal gene is operably linked to an endogenous inducible promoter.

37. A method as claimed in claim 35 ~~or claim 36~~ wherein the host cell is a mycolic acid bacterium of the same strain from which the inducible promoter and/or operon proteins were isolated.

38. A method of producing a biosensor comprising the method of <sup>claim 35</sup> ~~any one of claims 35 to 37~~.

39. A biosensor host transformed with a vector as claimed in <sup>claim 32</sup> ~~any one of claims 32 to 34~~ or as produced by the method claim 38.

40. A method of detecting the presence or absence of an analyte in a sample comprising the steps of:  
(a) contacting the sample with a transformed microorganism which is a mycolic acid bacterium which expresses a binding agent capable of binding the analyte, wherein the binding of the agent to the analyte causes a detectable signal, and wherein said bacterium has been transformed such as to improve the detectability of the signal; and  
(b) observing said bacterium for said detectable signal.

41. A method as claimed in claim 40 wherein the transformed microorganism is the biosensor of claim 39.

42. A method as claimed in claim 40 ~~or claim 41~~ wherein the signal is detected by an increased expression of a heterologous signal protein from a signal gene.

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Claim 40

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claim

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Claim 21

add A17

add C5

add D6

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